

Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm

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Anthropogenic climate change poses a serious threat to biodiversity. In marine environments, multiple climate variables, including temperature and CO₂ concentration ([CO₂]), are changing simultaneously. Although temperature has well-documented ecological effects, and many heavily calcified marine organisms experience reduced growth with increased [CO₂], little is known about the combined effects of temperature and [CO₂], particularly on species that are less dependent on calcified shells or skeletons. We manipulated water temperature and [CO₂] to determine the effects on the sea star *Pisaster ochraceus*, a keystone predator. We found that sea star growth and feeding rates increased with water temperature from 5 °C to 21 °C. A doubling of current [CO₂] also increased growth rates both with and without a concurrent temperature increase from 12 °C to 15 °C. Increased [CO₂] also had a positive but nonsignificant effect on sea star feeding rates, suggesting [CO₂] may be acting directly at the physiological level to increase growth rates. As in past studies of other marine invertebrates, increased [CO₂] reduced the relative calcified mass in sea stars, although this effect was observed only at the lower experimental temperature. The positive relationship between growth and [CO₂] found here contrasts with previous studies, most of which have shown negative effects of [CO₂] on marine species, particularly those that are more heavily calcified than *P. ochraceus*. Our findings demonstrate that increased [CO₂] will not have direct negative effects on all marine invertebrates, suggesting that predictions of biotic responses to climate change should consider how different types of organisms will respond to changing climatic variables.

calcification | climate change | feeding rate | ocean acidification | *Pisaster ochraceus*

Anthropogenic climate change poses one of the most serious threats to biodiversity. The emission of greenhouse gases and other human activities are already driving rapid change in the Earth's climate system, and rates of change are expected to accelerate in the current century (1). In some cases (e.g., ocean pH) environmental conditions are expected to exceed values that have not been reached in many millions of years (2). Although the ecological effects of anthropogenic climate change are already being felt (3, 4), the full repercussions for natural ecosystems and human societies remain poorly understood.

In marine environments, the 2 most important abiotic changes are likely to be increased water temperature and elevated carbon dioxide concentration ([CO₂]) (4). The mean global surface temperature has increased ≈0.76 °C in the past 150 years and is predicted to rise an additional 1°–4 °C by the end of this century (1). Both positive and negative biological responses to increased temperature have been well documented and include vertical and latitudinal range shifts, altered feeding and growth rates, and acute responses such as coral bleaching (5–8). Less studied are the biological effects of ocean acidification (OA), which collectively refers to increased oceanic [CO₂] and the corresponding reduction in pH and carbonate availability (2, 9). Seawater pH has dropped ≈0.1 units since the Industrial Revolution and is expected to decrease by another 0.15–0.35 units by the year 2100

(1). Furthermore, the reduction in carbonate availability, a component of calcium carbonate (CaCO₃) required by many marine calcifying organisms (2, 9), is believed to be a major driver of decreased growth rates in mollusks, gastropods, coccolithophorids, and other heavily calcified species with experimental increases in [CO₂] (10–12). However, the effects of ocean acidification on species that are less dependent on calcified shells or skeletons are poorly understood.

In addition to the important effects of individual climatic variables, simultaneous changes in multiple climate variables have the potential to yield surprising physical and biological responses that could not be predicted by responses to single climate variables alone. For instance, CO₂ solubility is temperature dependent, and therefore pH may show less extreme changes when increases in [CO₂] and temperature are combined (2). Similarly, multiple stressors may have synergistic, antagonistic, or additive effects on marine species (13–15). For example, elevated temperature and [CO₂] have synergistic negative effects on the calcification rates of tropical corals (14). Ocean acidification may also increase organisms' susceptibility to other stressors; elevated [CO₂] reduces thermal tolerance in crabs (16) and heat-shock protein production in echinoid larvae (17). To fully understand the biological consequences of climate change, multiple climate variables must be experimentally manipulated in tandem.

Pisaster ochraceus (Brandt, 1835), a primarily intertidal sea star, is an ideal study organism to address the aforementioned questions. The importance of temperature on *P. ochraceus* biology has already been established; upwelling-associated cooling of seawater has been shown to reduce *P. ochraceus* feeding rates and alter growth rates (7). However, the extent to which these biological rates will increase with incremental increases in water temperature and the limits to this increase remain unknown. *P. ochraceus* also presents an interesting test of the effects of OA on less-calcified members of a phylum that has so far shown primarily negative responses to OA. Rather than a continuous, heavily calcified endo- or exoskeleton, *P. ochraceus* instead has hundreds of tiny calcareous ossicles embedded within and connected by soft tissue (18, 19), and these ossicles make up a relatively small proportion of *P. ochraceus*' total body mass. Finally, *P. ochraceus* plays an important role in determining rocky intertidal community structure along the western coast of North America (6, 7, 20–22) and commonly feeds on heavily calcified species, such as mussels, that are predicted to experience reduced growth with OA (23, 24). If *P. ochraceus*' response to climate change differs substantially from its prey's response, this could have important implications for the strength of the predator–prey interaction. Overall community responses to cli-

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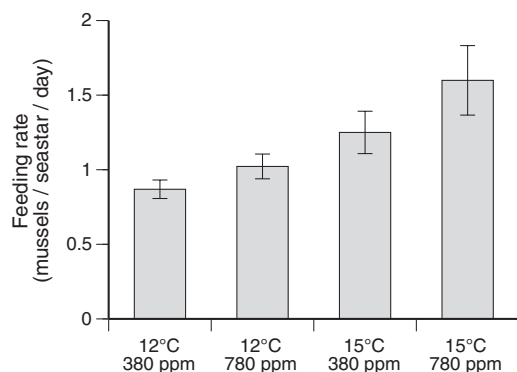


Fig. 3. Mean number of mussels consumed daily per sea star under 4 factorial temperature and [CO₂] combinations. Error bars represent ± 1 SE of the mean.

reduced in the high temperature treatments (Fig. 4; ANCOVA; temperature, $F_{1,17} = 2.21$, $P = 0.115$; CO₂, $F_{1,17} = 4.84$, $P = 0.042$; temp \times CO₂, $F_{1,17} = 4.84$, $P = 0.042$; sea star wet mass, $F_{1,17} = 8.48$, $P = 0.01$). Although the relative noncalcified wet mass [(dry soft tissue mass + water mass)/total wet mass] increased with [CO₂], the ratio of dry soft tissue mass to water mass remained constant at $\approx 1:4$ regardless of temperature or [CO₂] (2-way ANOVA; temperature, CO₂, and temp \times CO₂, all $P > 0.3$).

Our findings show similarities but also key differences from previous studies on the effects of climate change on marine organisms. Increased [CO₂] reduces calcification rates in a variety of marine invertebrates, leading to reduced growth rates (Table 1). Although we found that the relative calcified mass of sea stars declined with increased [CO₂], *P. ochraceus*' overall growth rate did not suffer as a consequence. This seeming disagreement with the responses of other marine invertebrates to elevated [CO₂] could be explained by differences in the amount and location of their calcareous tissue. Unlike urchins, mollusks, and brittle stars, *P. ochraceus* lacks a continuous calcified test, shell, or endoskeleton that encases a large portion of its soft tissue, making it less likely that a reduction in the growth of *P. ochraceus*' calcareous material would physically limit soft tissue growth or function (Table 1; ref. 19). Furthermore, *P. ochraceus*' calcified ossicles make up a relatively small proportion of its total body mass (R.G. and E.T., unpublished data). It may be that elevated [CO₂] decreased the rate at which *P. ochraceus* added calcareous material as it does in other species, but the lack of a continuous calcified shell or test in *P. ochraceus* allowed soft

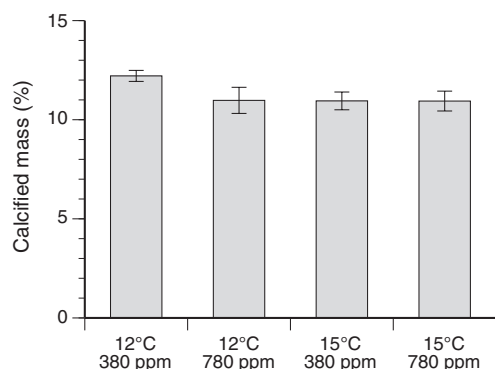


Fig. 4. Mean proportion of sea star wet mass consisting of calcified material under 4 factorial temperature and [CO₂] combinations. Error bars represent ± 1 SE of the mean. To account for the confounding effect of sea star size on feeding rate, data were adjusted to the approximate median sea star wet mass (12 g).

tissue growth to continue despite reduced calcification. Whatever the cause, we found no apparent negative effects of reduced calcification on the growth, feeding, and survival of *P. ochraceus* during our experiment. However, the long-term fitness consequences of reduced calcification in sea stars are unknown.

Despite the reduction in relative calcified mass with increased [CO₂], the overall effect of [CO₂] on growth was positive. The reasons for the observed increase in growth with elevated [CO₂] are somewhat unclear. The ratio of dry soft tissue mass to water mass remained unchanged by temperature or [CO₂], suggesting that the change in relative calcified mass must have been caused at least in part by an increase in the rate of wet soft tissue growth. Because we could not measure change in calcified mass over the course of the experiment, it is unclear whether the rate of calcified tissue growth simply remained the same as that of sea stars reared at control [CO₂] (thereby failing to keep pace with the increased soft tissue growth) or declined compared to that of control [CO₂] sea stars. Experiments specifically testing sea star calcification rates under control and high [CO₂] conditions will be necessary to answer this question.

Although the unchanged ratio of dry soft tissue mass to water mass demonstrates that the greater growth of sea stars reared at high [CO₂] was primarily because of increased wet soft tissue growth, it does not explain the mechanism behind this increase. The nonsignificant trend of increased feeding with increased [CO₂] suggests that although feeding rate may be partially responsible for the increase in growth rate, there are likely additional factors contributing to this change. It is possible that elevated [CO₂] increases resource use efficiency; for example, the slightly lower pH of high-CO₂ seawater could aid in the digestion of prey tissue, making feeding less energetically costly. Alternatively, low level stressors such as low doses of toxins can elicit positive responses such as increased growth in plants, invertebrates, and vertebrates, a phenomenon referred to as hormesis (26); the stress of reduced pH or carbonate availability may elicit a similar response in sea stars. Identification of the precise mechanism driving the increase in wet soft tissue growth with elevated [CO₂] will require further, more physiologically based experiments.

Several studies have found predominantly negative and non-additive effects of multiple climate variables on the growth and survival of marine organisms (13–15). The lack of a similar negative or synergistic response in *P. ochraceus* could be explained by the relative thermal tolerances and environments of sea stars vs. many previously studied species. For example, tropical corals often live at or near their thermal tolerance for water temperature (8) and are generally experimentally manipulated at or near these levels (e.g., ref. 14). Therefore, the effect of any additional stress may be magnified. *P. ochraceus*, in contrast, was well within its thermal range in our experiments and, as we have shown here, is unlikely to surpass its optimal water temperature with future climate change in much of its geographic range. Our findings also suggest that the nature of an interaction between climate variables depends on the response variable being measured, even for the same species. In the case of *P. ochraceus*, we found that temperature and [CO₂] had an antagonistic interaction in their effects on relative calcified material, whereas they had a positive and additive effect on overall growth rates. These within-species differences in the interaction between and effects of combined temperature and [CO₂] add an additional level of complexity when attempting to categorize the interactions between multiple climate variables.

Our findings suggest that caution should be exercised when predicting species' responses to climate change on the basis of broad phylogenetic relationships alone. Negative responses to OA in ophiuroids (brittle stars, ref. 27) and echinoids (sea urchins, ref. 11), both in the phylum Echinodermata, have led to overgeneralized predictions that echinoderms will respond neg-

for statistical analyses. Each sea star was housed in its own $8 \times 10 \times 10$ -cm plastic container with mesh sides and top to allow water to flow through. Two containers were then randomly assigned to each tank; there was no trend between the experimental temperature and initial sea star size (simple linear regression: $P > 0.1$, $df = 37$, $R^2 = 0.02$). At the beginning of the experiment, initial wet mass was measured to the nearest 0.01 g. For all wet mass measurements, each sea star was removed from the water, gently patted dry with a paper towel, immediately weighed on a scale, and then returned to the water. The sea stars acclimated in their assigned tanks at 12.8 °C for 4 days without food, then the tanks were changed to their experimental temperatures over an 8-h period, and finally the sea stars acclimated for an additional 6 days without food.

After the acclimation period, 20 small mussels (15 ± 2 mm shell length) were placed in each container. Empty shells were removed, recorded, and replaced with live mussels every other day. No sea star ran out of mussels during the course of the experiment. Wet mass was measured weekly. After 21 days of feeding, sea stars were wet weighed and all mussels were removed. To control for any effect of water temperature on water retention in the sea stars, tank temperatures were brought back to 12.8 °C over an 8-h period and sea stars reacclimated to this temperature for an additional 48 h without food before their final wet mass was measured.

Sea Star Growth and Feeding Rates with Temperature and [CO₂]. Juvenile sea stars (wet mass = 4.25 ± 0.10 g) were randomly assigned to 1 of 4 treatments: 12 °C and 380 ppm CO₂ ($n = 5$), 12 °C and 780 ppm CO₂ ($n = 6$), 15 °C and 380 ppm CO₂ ($n = 6$), and 15 °C and 780 ppm CO₂ ($n = 5$). These combinations were chosen to approximate current and predicted future levels of change by the year 2100 (Intergovernmental Panel on Climate Change IS92a emissions scenario). The tanks and containers were the same as those described in the previous experiment. Tanks were assigned to treatments using a stratified random design. Temperature was maintained using external tank chillers as above, while CO₂ concentrations were maintained using mass flow controllers to constantly bubble the tanks with either ambient air (containing ≈ 380 ppm CO₂) run through an air compressor or the appropriate mixture of compressed CO₂ (2% CO₂ with balance air; Praxair) and ambient air from an air compressor. The tanks were covered with lids to help maintain the desired [CO₂] in the tank headspace and seawater.

Two sea stars, each in their own container, were randomly assigned to each tank. The mean initial sea star wet mass did not differ between treatments (1-way ANOVA: $F_{3,40} = 1.02$, $P = 0.395$). Sea stars acclimated in their tanks without food for 9 days while the tanks equilibrated to experimental conditions. The initial wet weights were measured, and then sea stars were fed mussels (shell length = 17 ± 2 mm) ad libitum for the remainder of the

experiment. Tank temperatures were measured at least 3 times a week. Tank pH's were also measured frequently to ± 0.01 pH units with a portable pH meter (YSI 556-MPS) calibrated at the appropriate experimental temperatures (12 °C and 15 °C). The mean standard deviation of within-tank pH over time was ± 0.015 , which was far smaller than the SD between tanks as well as between treatments. The mean pH of each tank was used to determine treatment means. Sea stars were wet weighed weekly. After 10 weeks, final wet mass was measured. Sea stars were then dried to constant mass in an oven at 70 °C and placed in ≈ 125 mL of a 10% bleach solution for 48–72 h to remove their soft tissue. The solution was then vacuum filtered on No. 1 Whatman filter paper to collect the calcified material, which was then dried at 70 °C to constant mass and reweighed.

Statistical Analyses. To avoid pseudoreplication, all biological variables measured were averaged for the 2 sea stars in each tank, and these tank means were used in all statistical analyses. The only exceptions to this were the analyses to determine if the mean initial sea star wet weights were the same across all treatments; individual sea star wet weights were used to calculate these means in both experiments. In the temperature-only experiment, separate simple linear regressions were used to determine the effect of water temperature on relative sea star growth rate [(final grams wet mass – initial grams wet mass)/initial grams wet mass $\times 100$] and per capita feeding rate (number of mussels consumed daily per sea star). Although a second-order polynomial model was initially used to analyze the relationship between temperature and feeding rate, the polynomial term was nonsignificant. Therefore, we present a linear model in the results.

In the 2×2 factorial experiment, a 2-way ANOVA was also used to determine the effects of temperature and [CO₂] on relative sea star growth (percentage of gain relative to initial sea star wet mass). Separate ANCOVAs were used to determine the effects of temperature and [CO₂] on sea star feeding rates (number of mussels consumed daily per sea star), percentage of wet mass consisting of calcareous material, and the ratio of dry soft tissue mass to water mass. Because absolute sea star size can affect all of these variables, sea star wet mass was initially included as a covariate in all analyses. When size effects were nonsignificant ($P > 0.1$), they were removed from the analyses. Feeding rate and relative growth data for the factorial experiment were log transformed to equalize variances, and all data were analyzed using JMP 8 (SAS Institute).

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